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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/066,359	01/31/2002	Scot R. Weinberger	CiphBio-9	5296

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EXAMINER	
DAVIS, DEBORAH A	

ART UNIT	PAPER NUMBER
1641	

DATE MAILED: 11/02/2004

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/066,359	WEINBERGER ET AL.	
	Examiner	Art Unit	
	Deborah A Davis	1641	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on 7-26-04.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) 6,7 and 9-14 is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) 6-7, 9-14 is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

1. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on July 26, 2004 has been entered. Currently claims 6-7 and 9-14 are pending and under consideration.

Claim Rejections - 35 USC § 103

2. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

3. Claims 6 and 9-14 are rejected under 35 U.S.C. 103(a) as being unpatentable over William T. Hutchens (WO98/59362) in view of Dongre et al (Emerging tandem-mass-spectrometry techniques for the rapid identification of proteins, TIBTECH, Vol. 15, October 1997).

The instant claims are directed to a method for identifying a protein that is differentially displayed between two complex biologic samples using mass spectrometry. William T. Hutchens teaches methods for identifying analytes that are

differentially expressed between biological materials using desorption spectrometry (see abstract). The two samples are differentially displayed because the proteins can be expressed in different cell types being normal versus pathologic cancer cells. The method may indicate that a protein or other biomolecule is increased or decreased in expression, or is changed in some way based on different mass (page 63, lines 1-32). Claim 6, steps (b) through (c) is directed to fragmenting proteins in two samples and detecting protein fragments determining the identity and correlating the fragments that are differentially displayed between the two samples. William T. Hutchens teaches that the fragmenting of large proteins into smaller pieces by enzymatic digestion increases sensitivity in detection of protein fragments. Fragmentation can be achieved by any means known in the art; some examples are enzymes such as glycosidase, endoproteases (page 64, lines 28-32). William T. Hutchens teaches proteins that are differentially present in two samples will increase the number of signals from that protein (page 64, lines 11-24). William T. Hutchens teaches that these methods of protein identification are useful for identifying diagnostic markers of disease expressed in a patient sample or a diseased cultured cell compared to normal samples (page 64, lines 1-10). Maps of the protein samples are compared, which may indicate increased or decreased expression in a protein (page 63, lines 22-32). Accordingly, the matched parameters can be set to identify the closeness-of-fit between the protein analyte characteristics and the characteristics of the reference polypeptides in the database (page 61, lines 15-31). William T. Hutchens' method further includes a capture probe to capture proteins. William T. Hutchens' instant reference teaches probes for the specific

detection of one or more analytes by desorption spectrometry, which can be prepared by selecting markers (candidates) to be detected (page 59, lines 19-33). The reference of Hutchens teaches the use of a Retentate chromatography to separate multiple analytes in parallel (page 52, lines 27-33).

The instant reference of William T. Hutchens does not teach utilizing the method with tandem mass spectrometry; neither does it teach steps of a secondary fragmentation step to generate parent peptides with a gas phase.

However, Dongre et al provides an overview of techniques and methodologies for identification of proteins and peptides from complex biological samples utilizing tandem mass spectrometry (see abstract). Tandem mass spectrometry is commonly used for sequence analysis of peptides and proteins that include techniques such as Collision Induced Dissociation (CID) that involves the collision of peptide ions in a gas phase at low speeds with an inert gas such as argon. The fragment ions that are generated from the gas collision upon peptide-ion activation, are then analyzed by a second mass analyzer (page 419, column 1 and Figure 1). The correct amino acid sequence is frequently identified purely on the basis of the preliminary score and a closeness-to-fit method is used to confirm the highest-scoring amino acid sequence and increase the sensitivity of the search (page 423, column 1, paragraph 1).

It would have been obvious to one of ordinary skill in the art to modify the reference of William T Hutchens to include tandem mass spectrometry and a secondary fragmenting step for generating peptides using gas as taught by Dongre et al because tandem mass spectrometry has several advantages. First, identification is possible on

the basis of a single peptide spectrum. Second, each tandem mass spectrum represents an independent piece of information, and so additional spectra that match the same protein add considerable strength to the identification. Third, the ability to identify proteins based on a single tandem mass spectrum allows the identification of proteins present in complex mixtures. Finally, post-translational modification do not appear to complicate the identification and can be placed within the amino acid sequence at the specific site of modification with the aid of computer programs (page 424, column 2, paragraph 2). Utilizing a secondary fragmenting step for generating peptides is also a method utilized by tandem mass analyses. This step is an advantage for tandem mass spectrometry because under low and high-energy gas-phase collision induced dissociation (CID) conditions, peptide ions that are generated mostly fragment at the peptide bonds along the backbone, generating a ladder of sequence ions. This information dictates which type of amino acid sequence will form and have lead to sequencing methods which is important when predicting peptide-fragmentation patterns (page 419, column 1).

4. Claims 6, and 9-14 are rejected under 35 U.S.C. 103(a) as being unpatentable over Liebler et al (USP# 6,379,970) in view of Dongre et al (Emerging tandem-mass-spectrometry techniques for the rapid identification of proteins, TIBTECH, Vol. 15, October 1997).

The instant claims are directed to a method for identifying a protein that is differentially displayed between two complex biologic samples using mass spectrometry.

Liebler et al teaches a method for detecting peptide fragments of protein(s) that are differentially present in biological samples. The identity of the peptides may be determined and correlated with the protein(s) that are differentially present in the samples (see abstract). Claim 6, steps (b) through (c) is directed to fragmenting proteins in two samples and detecting protein fragments determining the identity and correlating the fragments that are differentially displayed between the two samples. Liebler et al teaches protein fragmentation wherein the proteins are digested in a plurality of biological samples to produce peptides in each sample; separating the peptides in the samples and identifying the peptides that are differentially present. The proteins contained in the biological samples may be digested with any of the well-known protein digestions reagents. Such reagents may be chemical or enzymatic (col. 5, lines 15-26 and col. 10, lines 1-20). The instant claim 6 utilizes mass spectrometry for detection of peptide and proteins. Liebler et al teaches that a variety of mass spectrometry techniques are routinely used to determine peptide sequence. Two MS ionization methods used in the field of protein analysis are electrospray ionization (ESI) and matrix assisted laser desorption ionization (MALDI). Both methods are effective means of producing gas phase ions of proteins peptides and other biomolecules for MS analysis (col. 7 lines 30-65). One non-limiting embodiment of the present invention involves the analysis of two peptide mixtures together in one analytical run. Once the

mixtures are combined and then subjected to some analytical separation the differential expression of the precursor protein are then selected for further analysis by mass spectrometry (col. 4, lines 32-45). Correlation of differentially produced peptides with differentially expressed proteins is performed by using amino acid sequences of signature peptides against a database of protein sequences (col. 8, lines 38-67). Samples may comprise of cultured cells, blood samples, biopsy or other biological fluids.

Liebler et al does not particularly point out using tandem mass spectrometry as recited in claim 6.

However, Dongre et al provides an overview of techniques and methodologies for identification of proteins and peptides from complex biological samples utilizing tandem mass spectrometry (see abstract). Tandem mass spectrometry is commonly used for sequence analysis of peptides and proteins that include techniques such as Collision Induced Dissociation (CID) that involves the collision of peptide ions in a gas phase at low speeds with an inert gas such as argon. The fragment ions generated from the gas collision, upon peptide-ion activation are then analyzed by a second mass analyzer (page 419, column 1 and Figure 1). The correct amino acid sequence is frequently identified purely on the basis of the preliminary score and a closeness-to-fit method is used to confirm the highest-scoring amino acid sequence and increase the sensitivity of the search (page 423, column 1, paragraph 1).

It would have been obvious to one of ordinary skill in the art to modify the reference of Liebler et al to include tandem mass spectrometry and a secondary

fragmenting step for generating peptides using gas as taught by Dongre et al because tandem mass spectrometry has several advantages. First, identification is possible on the basis of a single peptide spectrum. Second, each tandem mass spectrum represents an independent piece of information, and so additional spectra that match the same protein add considerable strength to the identification. Third, the ability to identify proteins based on a single tandem mass spectrum allows the identification of proteins present in complex mixtures. Finally, post-translational modification do not appear to complicate the identification and can be placed within the amino acid sequence at the specific site of modification with the aid of computer programs (page 424, column 2, paragraph 2). Utilizing a secondary fragmenting step for generating peptides is also a method utilized by tandem mass analyses. This step is an advantage for tandem mass spectrometry because under low and high-energy gas-phase collision induced dissociation (CID) conditions, peptide ions that are generated mostly fragment at the peptide bonds along the backbone, generating a ladder of sequence ions. This information dictates which type of amino acid sequence will form and have lead to sequencing methods which is important when predicting peptide-fragmentation patterns (page 419, column 1).

5. Claim 7 is rejected under 35 U.S.C. 103(a) as being unpatentable over William T. Hutchens in view of Dongre et al and further in view of Little et al (USP#6,322,970).

The teachings of William T. Hutchens in view of Dongre et al are set forth above and differ from the instant claim in not specifically pointing out analyzing capture proteins on a probe.

However, Little et al teaches methods of detecting polypeptides using mass spectrometry. Little et al teaches using a microchip to isolate a polypeptide as well as a means to manipulate the isolated target polypeptide prior to mass spectrometry. In particular embodiments, post-translational capture and immobilization of a target polypeptide are provided in order to sequence a polypeptide. This method includes immobilizing the target polypeptide to a solid surface and cleaving the fragments with enzymatic treatment, which will improve mass spectrometric analysis (col. 4, lines 24-67 and col. 6, lines 10-15).

It would have been obvious to one of ordinary skill in the art to modify the teachings of William T. Hutchens in view of Dongre et al to include a microchip as taught by Little et al to capture proteins fragments because it will improve mass spectrometric analysis of protein fragments.

Response to Arguments

6. Applicant argues that the prior art does not teach step (e), which requires correlating the candidate for the protein cleavage product of step (d) with the differentially displayed protein of step (b). This argument is not found persuasive.

In response to applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986). Although the reference of Hutchens is silent with respect to teaching utilizing the

method with tandem mass spectrometry, in combination with LDI for further analysis of the cleavage products, the reference of Dongre teaches why one of ordinary skill in the art would be motivated to further analyze the protein cleavage products of step (d) and correlate its findings with step (b). Hutchens taught separation of proteins and peptides cleavage utilizing Chromatography and other separation procedures in combination with LDI, followed with mass spectral analysis (page 34, lines 32-33, and page 35, lines 1-.30) and identified by database mining to match parameters. Dongre taught HPLC in combination with electrospray ionization to separate and cleave protein/ peptide fragments and receive spectral information about the peptides (see page 420, Figure 2). Further analysis was made with tandem mass spec data (see page 420, Figure 2). Tandem mass spec is commonly used to obtain sequence analysis of a peptide ions and proteins (page 419, 1st paragraph, column 1). Therefore, the further analysis with tandem mass spec offers considerable strength in identifying proteins or peptides. Further, tandem mass spec, in combination with other mass analyzers to further confirm the identity of a protein or peptide are standard procedures that are known to one of ordinary skill in the art. Therefore, it is the examiner's position that the combination of Hutchens and Dongre is proper and is obvious over the instant claimed method.

7. Applicant's appears to being arguing that the reference of Liebler taught a method of analyzing a protein starting out with proteolytic cleavage products without antecedent analysis of the differentially displayed biomarker where there would have

been no way to have known which among the identity candidates was the biologically relevant form. This argument is not found to be persuasive.

In reponse to applicant argument, the reference of Liebler taught the identity of peptide fragments of protein(s) that can be determined and correlated with the proteins that are differentially present in the samples (see abstract). Therefore, the identity of the original protein from the sample can be correlated and determined by its fragments.

8. Applicant argues that the reference of Liebler omits steps (a) and (b), beginning with the analysis of proteolytic digestion. This argument is not found to be persuasive.

In reference to applicant argument, steps (a) and (b) are taught by the reference of Liebler (see abstract, column 5, lines 15-26 and column 10, lines 1-20). With respect to step (a) of claim 6 that makes reference to absorbing proteins on the surface of an affinity capture probe, the reference of Liebler conducts mass spectra using matrix assisted laser desorption ionization or MALDI in the analysis of a protein fragments wherein this procedure involves absorbing peptides on an inert probe surface (column 7, lines 30-65), which reads on applicant's affinity probe of step (a).

9. Applicant broadly (page11) argues that the instant invention provides several phases of a mass analysis of a large protein and fragmenting it down to a smaller protein in an effort to correctly identify a potential biomarker. Unlike the prior art of Hutchens which taught analysis of the biomarker after only the first phase of fragmentation which would have been incorrect. This argument is not found persuasive.

In response, applicant's arguments fail to comply with 37 CFR 1.111(b) because they amount to a general allegation that the claims define a patentable invention without specifically pointing out how the language of the claims patentably distinguishes them from the references. The identification of a biomarker is not found in the claims and mere repetition of steps such as fragmentation of larger proteins down to smaller proteins for further analysis are known to one of ordinary skill in the art. Absent evidence to the contrary, it is the examiner's position that the prior art still apply.

Conclusion

10. No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Deborah A Davis whose telephone number is (571) 272-0818. The examiner can normally be reached on 8-5 Monday thru Friday.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Long Le can be reached on (571) 272-0823. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

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10/27/04